IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:

R. S. OBACH

APPLICATION NO.: 09/528798

FILING DATE: March 21 2000

TITLE: USE OF A CYP2D6 INHIBITORS IN COMBINATION THERAPIES

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. 1.132

Sir:

RONALD SCOTT OBACH, hereby declares, states and says that:

1. He received a B.S. from the State University of New York at Binghamton in 1985, and a Ph.D. from from Brandeis University in 1990.

2. He is currently employed by Pfizer Inc. as a Research Advisor in the Pfizer research facility in Groton, Connecticut, and he has worked at Pfizer Inc. for 11 years.

3. He is familiar with the subject matter of the above-identified application and the references cited therein.

4. The above-identified application is directed to a method of administering the drug (2S,3S)-2-phenyl-3-(2-methoxy-5-trifluoromethoxyphenyl)methylamino-piperidine, or a pharmaceutically acceptable salt thereof, in combination with a CYP2D6 inhibitor, or a pharmaceutically acceptable salt thereof, to a human in need of the intended pharmaceutical activity of the drug, wherein the drug and the CYP2D6 inhibitor are not the same compound.

The CYP2D6 inhibitor may be, for example, quinidine, ajmalacine or pharmaceutically acceptable salts thereof.

5. In the enclosed data for the compound (2S,3S)-2-phenyl-3-(2-methoxy-5-trifluoromethoxyphenyl)methylamino-piperidine, denoted as "CP-B" in the data, Tables 1-4 describe enzymatic kinetic parameters for the metabolism of the compound (including O-demethylation and N-dealkylation) in various mammals, and Table 5 describes the inhibition of the same compound by Cytochrome P450 isoform specific inhibitors. In the figures, Figures 10 and 11 show a correlation between metabolism and inhibition of the same compound using quinidine (Figure 10) and ketoconazole (Figure 11).

6. The foregoing data and figures show a surprising effectiveness of (2S,3S)-2-phenyl-3-(2-methoxy-5-trifluoromethoxyphenyl)methylamino-piperidine in combination with a CYP2D6 inhibitor such as, for example, quinidine or ketoconazole.

He further declares that all statements made herein of his own knowledge are true and all statements made on information and belief are believed to be true. All statements made herein are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under section 1001 of Title 18 of the United States Code, and that willful false statements may jeopardize the validity of the above application or any patent that may issue from it.

Date: 03-Dec-2003

Ronald Scott Obach

TABLE 1. ENZYME KINETIC PARAMETERS FOR METABOLISM OF CP- B IN POOLED HUMAN LIVER MICROSOMES

Parameter	(O-demethylation)	(N-dealkylation)
Kinetic Behavior	simple	sigmoidal
K_{Mapp} (μM)	0.24	30
V_{max} (pmol/min/mg)	14	150
CL'_{int} (μL/min/mg)	59	5.2
Hill Coefficient	--	1.5
$K_{M(free)}$ (μM)	0.041	5.1
$CL'_{int(free)}$ (μL/min/mg)	350	31
scaled CL'_{int} (mL/min/kg)[a]	53	4.7
scaled $CL'_{int(free)}$ (mL/min/kg)[a]	320	28

[a]Intrinsic clearance scaled per kg body weight using the values of 45 mg microsomal protein per gm liver and 20 gm liver per kg body weight in human.

"free" parameters are corrected for $f_{u(microsomes)} = 0.166$

TABLE 2. ENZYME KINETIC PARAMETERS FOR METABOLISM OF CP. B IN POOLED RAT LIVER MICROSOMES

	(O-demethylation)		(N-dealkylation)	
Parameter	Male	Female	Male	Female
Kinetic Behavior	sigmoidal/ substrate inhibition	sigmoidal/ substrate inhibition	biphasic	sigmoidal/ biphasic
K_{Mapp} (μM)	0.44	0.36	43	88
V_{max} (pmol/min/mg)	270	120	68	33
$CL'_{int(1)}$ (μL/min/mg)	610	330	1.6	0.40
$CL'_{int(2)}$ (μL/min/mg)	--	--	0.20	0.22
$CL'_{int(total)}$ (μL/min/mg)	610	330	1.8	0.62
K_{iapp} (μM)	3.6	2.3	--	--
Hill Coefficient	1.5	1.7	--	4.3
$K_{M(free)}$ (μM)	0.070	0.047	6.9	11
$K_{iapp(free)}$ (μM)	0.58	0.30	--	--
$CL'_{int(total, free)}$ (μL/min/mg)	3800	2500	11	4.8
scaled CL'_{int} (mL/min/kg)[a]	1100	590	3.2	1.1
scaled $CL'_{int(free)}$ (mL/min/kg)[a]	6800	4500	20	8.6

[a]Intrinsic clearance scaled per kg body weight using the values of 45 mg microsomal protein per gm liver and 40 gm liver per kg body weight in rat.

"free" parameters are corrected for $f_{u(microsomes)}$= 0.130 for female, 0.160 for male

CP-B

TABLE 3. ENZYME KINETIC PARAMETERS FOR METABOLISM OF IN POOLED DOG LIVER MICROSOMES

Parameter	(O-demethylation)	(N-dealkylation)	N-Hydroxylation
Kinetic Behavior	simple	sigmoidal	substrate inhibition
K_{Mapp} (μM)	1.4	110	170
V_{max} (pmol/min/mg)	210	240	880
CL'_{int} (μL/min/mg)	140	2.2	5.2
K_{iapp} (μM)	--	--	690
Hill Coefficient	--	1.5	--
$K_{M(free)}$ (μM)	0.20	15	24
$K_{iapp(free)}$ (μM)	--	--	97
$CL'_{int(free)}$ (μL/min/mg)	1000	16	37
scaled CL'_{int} (mL/min/kg)[a]	200	3.2	7.5
scaled $CL'_{int(free)}$ (mL/min/kg)[a]	1400	23	53

[a]Intrinsic clearance scaled per kg body weight using the values of 45 mg microsomal protein per gm liver and 32 gm liver per kg body weight in dog.

"free" parameters are corrected for $f_{u(microsomes)}$= 0.144

TABLE 4. ENZYME KINETIC PARAMETERS FOR METABOLISM OF CP-B IN POOLED MONKEY LIVER MICROSOMES

Parameter	(O-demethylation)	(N-dealkylation)
Kinetic Behavior	simple	complex
K_{Mapp} (μM)	0.73	80
V_{max} (pmol/min/mg)	98	620
$CL'_{int(1)}$ (μL/min/mg)	130	7.7
$CL'_{int(2)}$ (μL/min/mg)	--	150
$CL'_{int(total)}$ (μL/min/mg)	130	160
K_{iapp} (μM)	--	52
Hill Coefficient	--	1.1
$K_{M(free)}$ (μM)	0.15	16
$K_{iapp(free)}$ (μM)	--	10
$CL'_{int(total, free)}$ (μL/min/mg)	650	800
scaled CL'_{int} (mL/min/kg)[a]	190	230
scaled $CL'_{int(free)}$ (mL/min/kg)[a]	940	1200

[a]Intrinsic clearance scaled per kg body weight using the values of 45 mg microsomal protein per gm liver and 32 gm liver per kg body weight in monkey.

"free" parameters are corrected for $f_{u(microsomes)}$= 0.198

TABLE 5. INHIBITION OF HUMAN LIVER MICROSOMAL CP-B METABOLISM BY CYTOCHROME P450 ISOFORM SPECIFIC INHIBITORS

Inhibitor	(O-demethylation)	(N-dealkylation)
quinidine		
IC_{50} (μM)	0.14	ND
maximum inhibition (%)	100	ND
ketoconazole		
IC_{50} (μM)	ND	0.076
maximum inhibition (%)	ND	100

ND, not determined

TABLE 6. CORRELATION OF CP- B METABOLISM TO CYTOCHROME P450 SPECIFIC MARKER ACTIVITIES IN A PANEL OF HUMAN LIVER MICROSOMES

Cytochrome P450 Specific Marker Activity	Correlation Coefficient (r^2) (O-demethylation)	(N-dealkylation)
phenacetin O-deethylase (CYP1A2)	0.003	0.002
tolbutamide hydroxylase (CYP2C9)	0.575	0.003
S-mephenytoin hydroxylase (CYP2C19)	0.094	0.074
bufuralol 1'-hydroxylase (CYP2D6)	0.863	0.001
testosterone 6ß-hydroxylase (CYP3A)	0.071	0.870[a]

a. Excludes one outlier point.

TABLE 7. METABOLISM OF CP-[illegible]B[illegible] BY HETEROLOGOUSLY EXPRESSED RECOMBINANT HUMAN CYTOCHROME P450 ENZYMES

CYP Enzyme	O-Demethylation (pmol/min/nmol CYP) [S] = 0.2 μM	N-Dealkylation (pmol/min/nmol CYP) [S] = 25 μM
CYP1A1	37	480
CYP1A2	ND	ND
CYP2A6	ND	ND
CYP2B6	ND	ND
CYP2C9	ND	ND
CYP2C19	ND	ND
CYP2D6	4400	ND
CYP2E1	ND	ND
CYP3A4	ND	4500
CYP3A5	ND	9400

ND = None Detected

TABLE 8. ENZYME KINETIC PARAMETERS FOR CP-β METABOLISM BY HETEROLOGOUSLY EXPRESSED RECOMBINANT HUMAN CYTOCHROME P450 ENZYMES

	rCYP2D6 O-Demethylation	rCYP3A4 N-Dealkylation	rCYP3A5 N-Dealkylation
Kinetic Behavior	simple	substrate inhibition	substrate inhibition
K_{Mapp} (μM)	0.057	11	22
V_{max} (nmol/min/nmol CYP)	0.36	19	67
CL'_{int} (mL/min/nmol CYP)	6.5	1.7	3.0
$K_{I(ap)}$ (μM)	--	1200	3800
$K_{M(free)}$ (μM)	0.041	1.4	3.7
$CL'_{int(free)}$ (mL/min/nmol CYP)	9.0	13	18
$K_{I(free)}$ (μM)	--	160	650

"free" parameters are corrected for $f_{u(microsomes)}$= 0.715, 0.133, and 0.173 for CYP2D6, 3A4, and 3A5, respectively.

TABLE 9. NON-SPECIFIC BINDING OF CP- B TO LIVER MICROSOMES AND RECOMBINANT CYP MICROSOMES.

Replicate	Matrix	Non-specific Binding % Bound[1]	% Free	Replicate	Matrix	Non-specific Binding % Bound	% Free
1	HL-Mix-12	84.5	15.5	1	RL-137 (male)	84.9	15.1
2	HL-Mix-12	82.3	17.7	2	RL-137 (male)	83.2	16.8
	Mean	83.4	16.6		Mean	84.1	16.0
	S.D.	1.6	1.6		S.D.	1.2	1.2
1	Dog Mix	83.2	16.8	1	rCYP3A4	86.8	13.2
2	Dog Mix	88.0	12.0	2	rCYP3A4	86.7	13.3
	Mean	85.6	14.4		Mean	86.8	13.3
	S.D.	3.4	3.4		S.D.	0.1	0.1
1	Monkey Mix	80.7	19.3	1	rCYP3A5	83.4	16.6
2	Monkey Mix	79.7	20.3	2	rCYP3A5	82.0	18.0
	Mean	80.2	19.8		Mean	82.7	17.3
	S.D.	0.7	0.7		S.D.	1.0	1.0
1	RL-129 (female)	88.2	11.8	1	rCYP2D6	25.7	74.3
2	RL-129 (female)	85.9	14.1	2	rCYP2D6	31.4	68.6
	Mean	87.1	13.0		Mean	28.6	71.5
	S.D.	1.6	1.6		S.D.	4.0	4.0

[1] Non-specific binding calculated as the ratio of (microsomes - buffer) concentration divided by the microsome concentration

Microsomal Protein Concentration is 0.5 mg/mL for all human and animal species.
rCYP protein concentration is 2.0 mg/mL for CYP3A4 and 3A5, and 0.026 mg/mL for CYP2D6

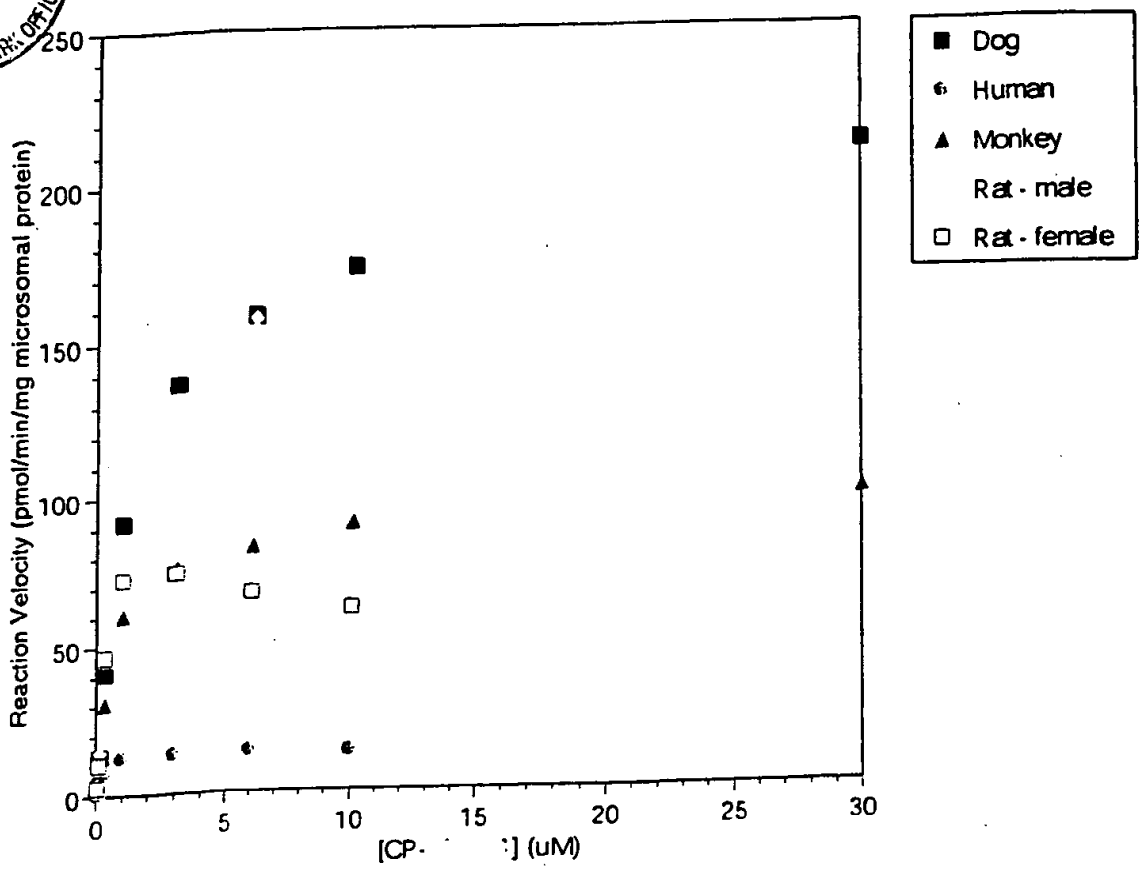

FIGURE 4. SUBSTRATE SATURATION CURVES FOR CP-B O-DEMETHYLATION IN LIVER MICROSOMES

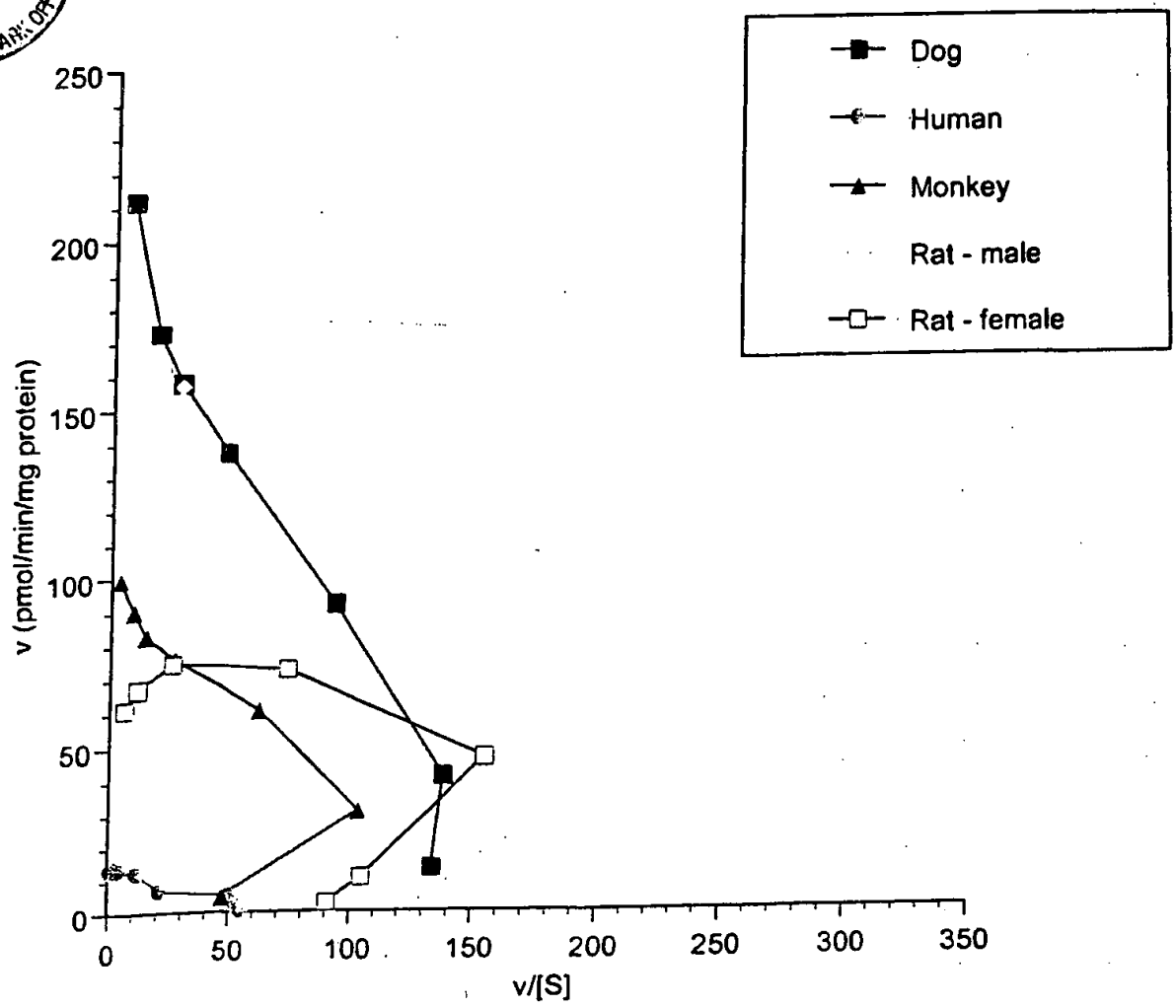

FIGURE 5. EADIE-HOFSTEE PLOT FOR CP-B O-DEMETHYLATION IN LIVER MICROSOMES

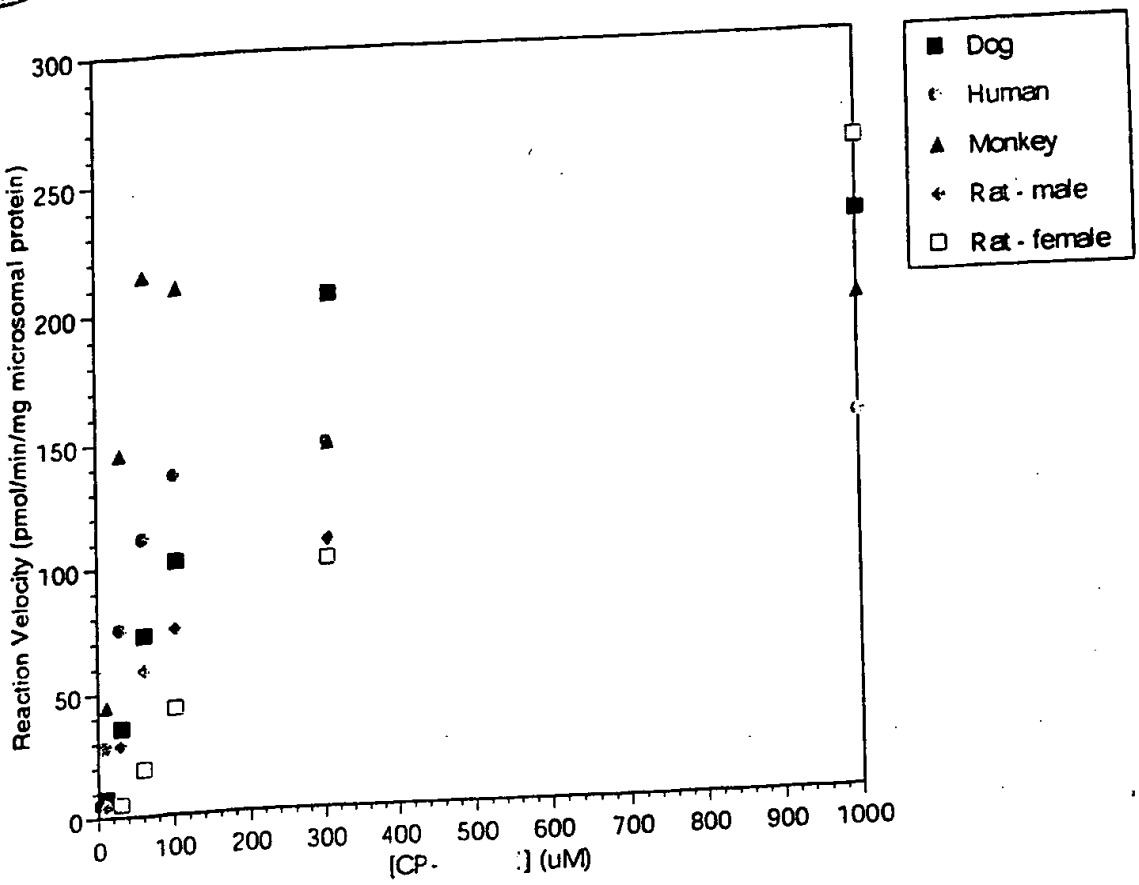

FIGURE 6. SUBSTRATE SATURATION CURVES FOR CP β N-DEALKYLATION :IN LIVER MICROSOMES

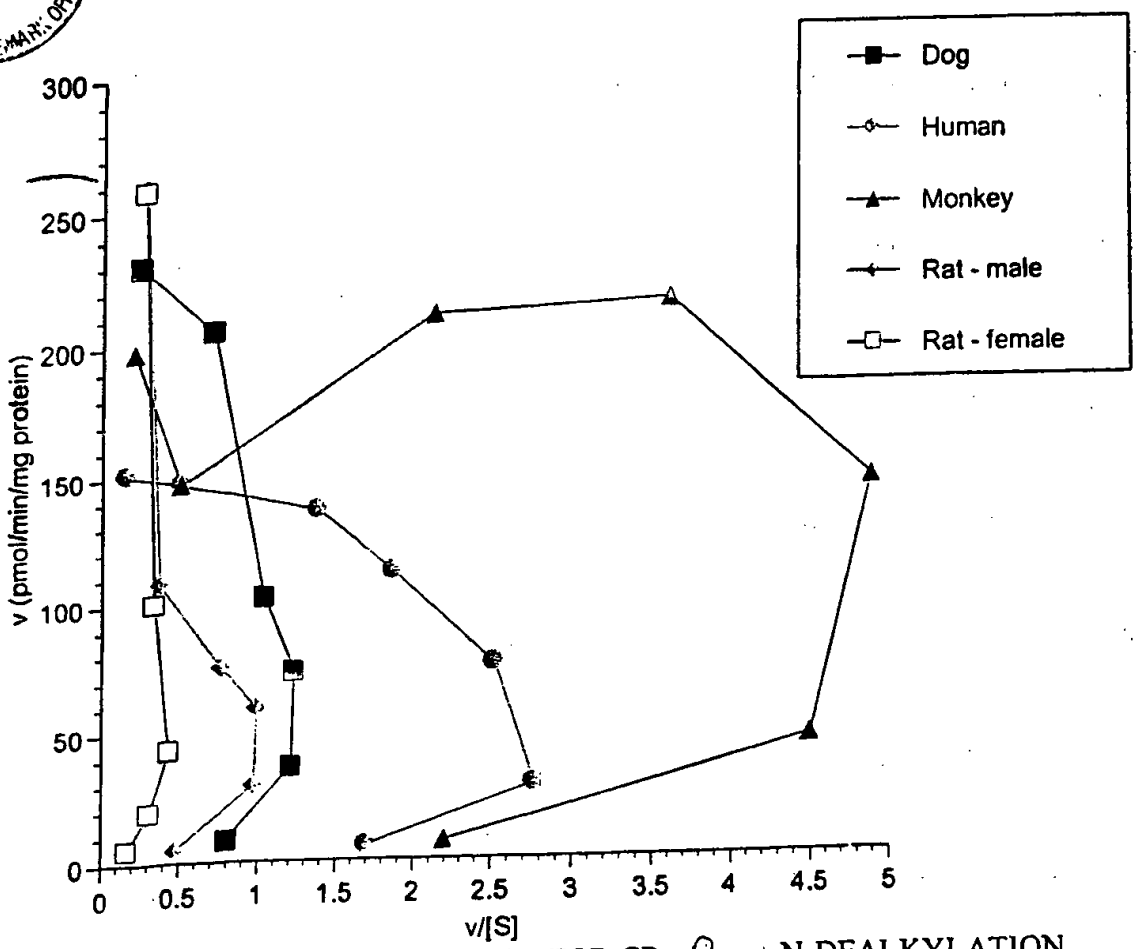

FIGURE 7. EADIE-HOFSTEE PLOT FOR CP-β N-DEALKYLATION KINETICS IN LIVER MICROSOMES

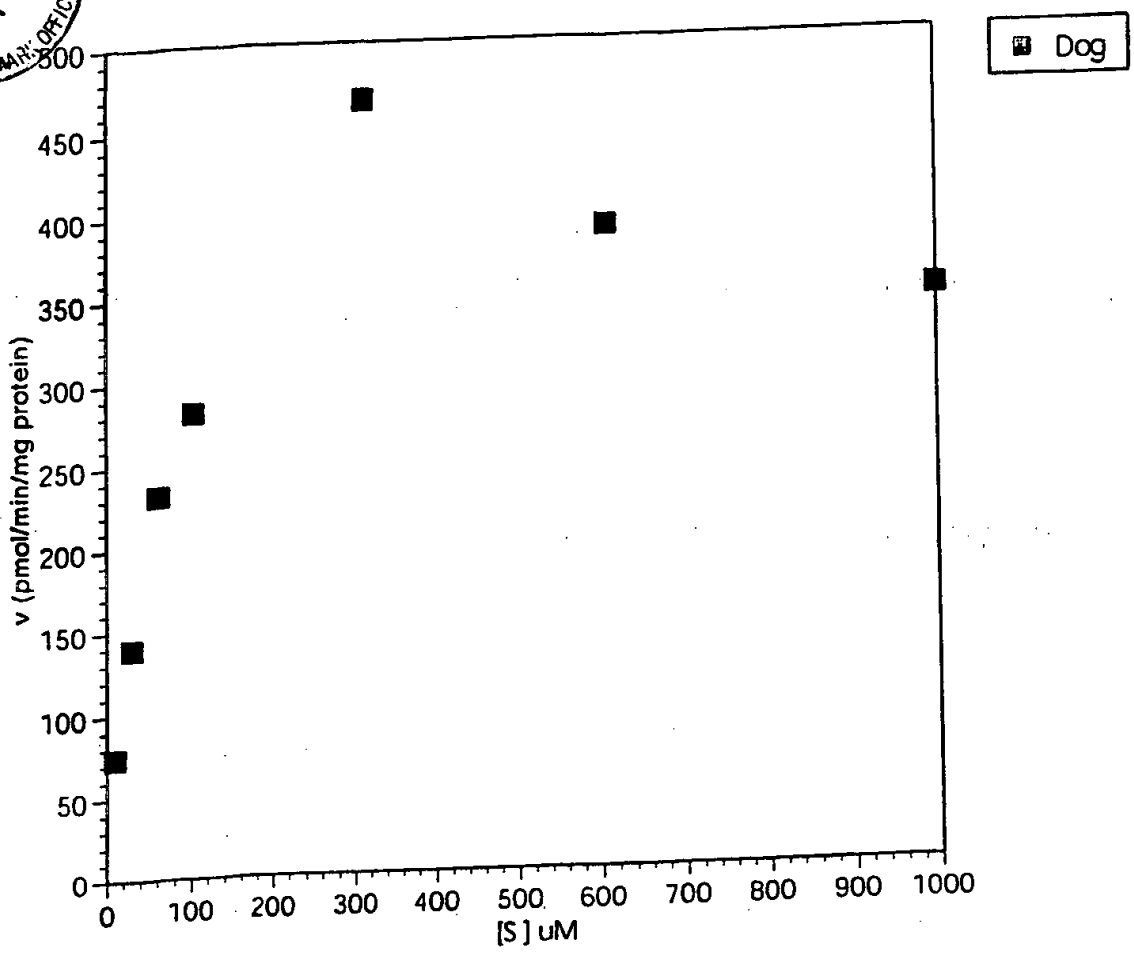

FIGURE 8. SUBSTRATE SATURATION CURVES FOR CP-β N-HYDROXYLATION IN DOG LIVER MICROSOMES

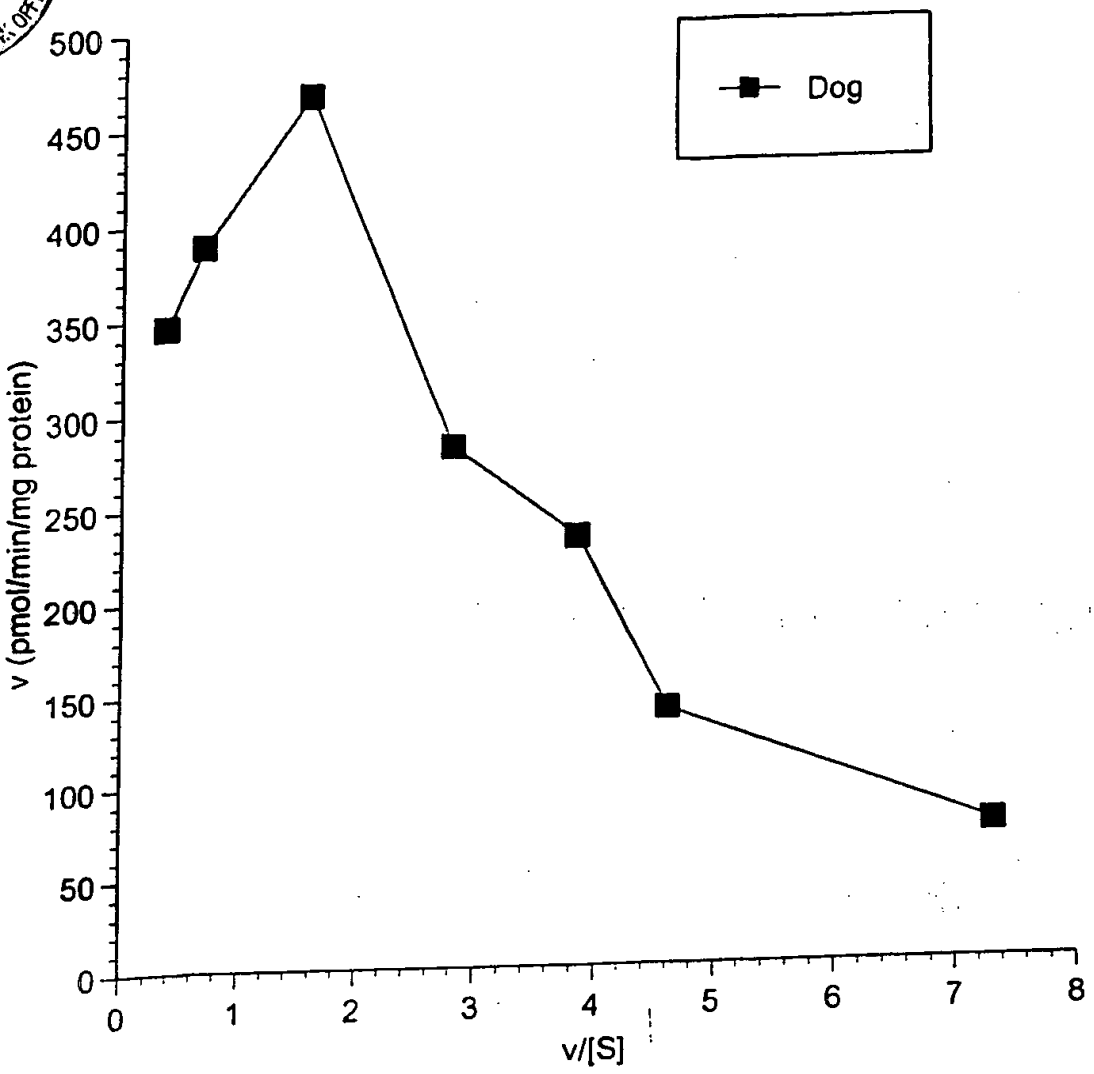

FIGURE 9. EADIE-HOFSTEE PLOT FOR CP- β N-HYDROXYLATION KINETICS IN DOG LIVER MICROSOMES

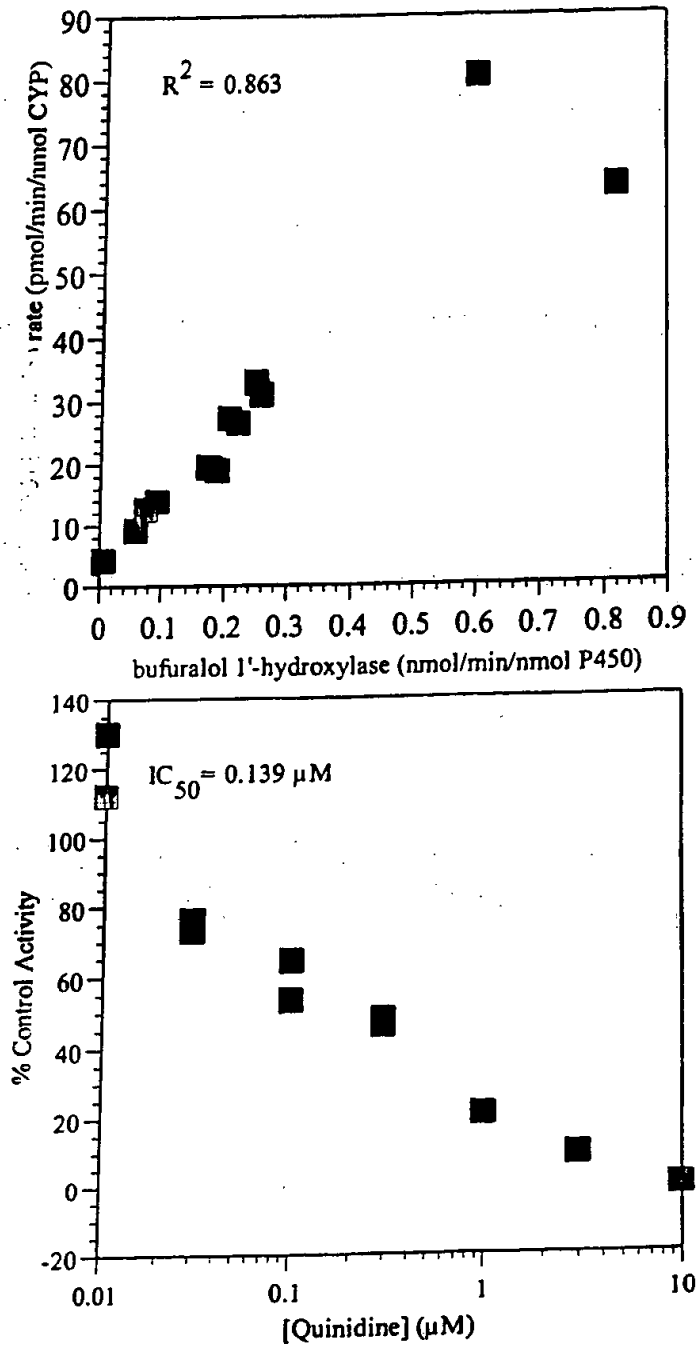

FIGURE 10. CP-B O-DEMETHYLATION: CORRELATION BETWEEN O-DEMETHYLATION AND BUFURALOL 1'HYDROXYLASE ACTIVITIES IN HUMAN LIVER MICROSOMES (TOP) AND INHIBITION OF CP-B O-DEMETHYLATION USING QUINIDINE, A CYP2D6 SPECIFIC INHIBITOR IN HUMAN LIVER MICROSOMES (BOTTOM).

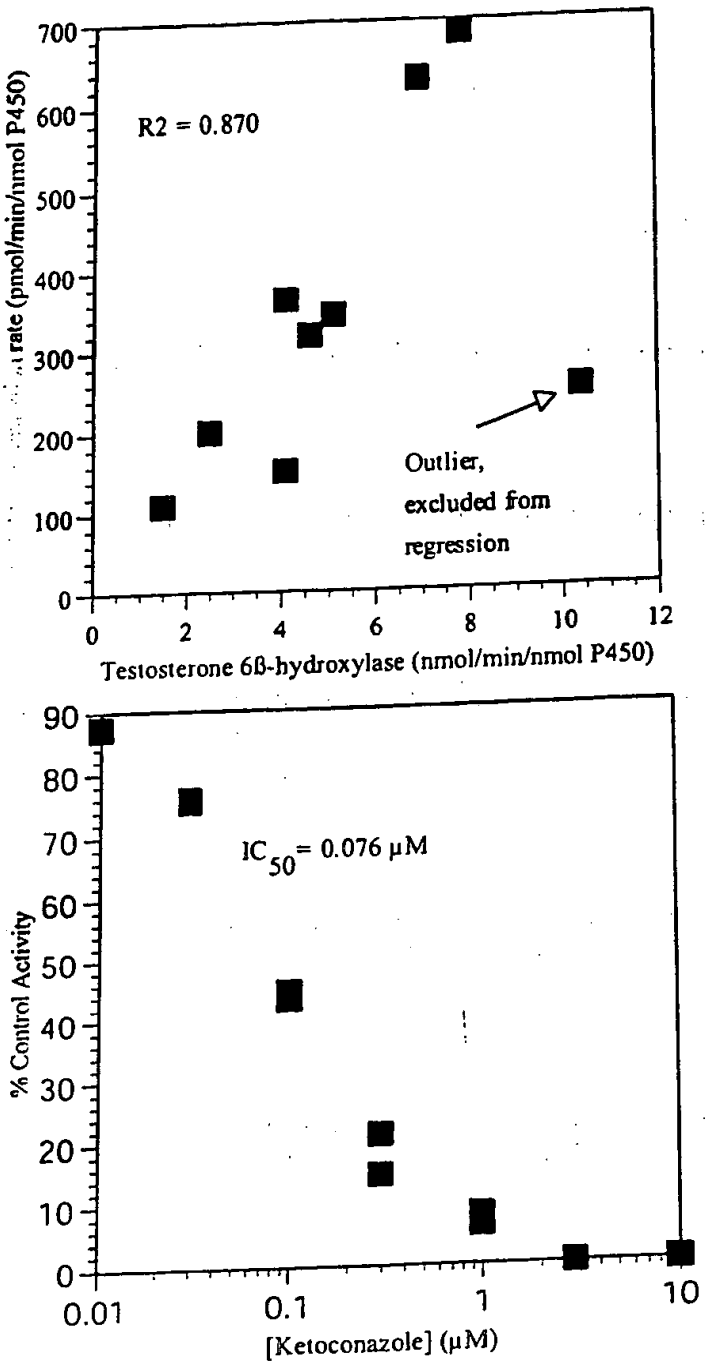

FIGURE 11. CP-B N-DEALKYLATION: CORRELATION BETWEEN N-DEALKYLATION AND TESTOSTERONE 6β-HYDROXYLASE ACTIVITIES IN HUMAN LIVER MICROSOMES (TOP) AND INHIBITION OF CP-B N-DEALKYLATION USING KETOCONAZOLE (A CYP3A SELECTIVE INHIBITOR) IN HUMAN LIVER MICROSOMES (BOTTOM).

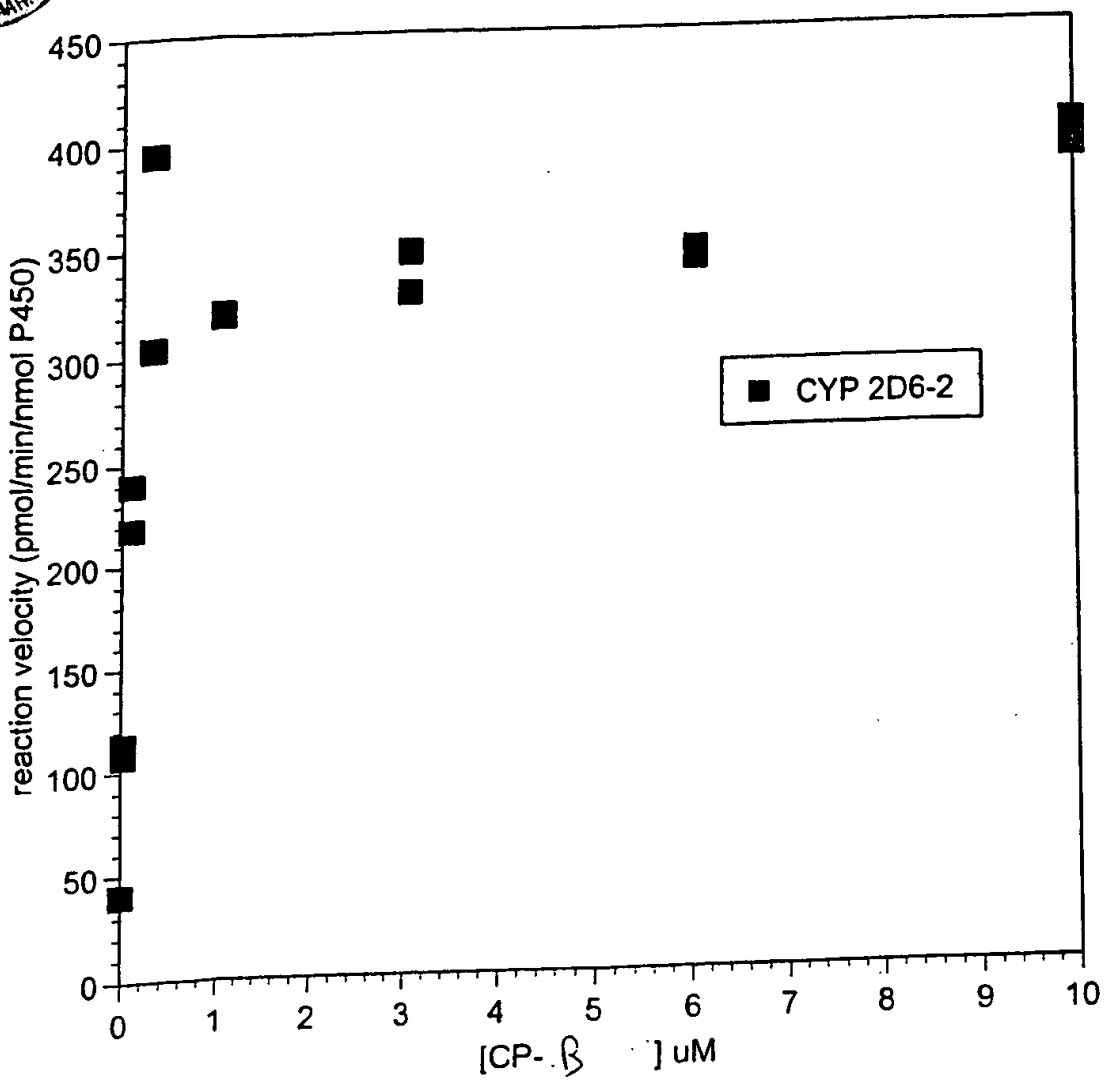

FIGURE 12. SUBSTRATE SATURATION PLOT OF CP-β O-DEMETHYLATION BY CYTOCHROME P4502D6

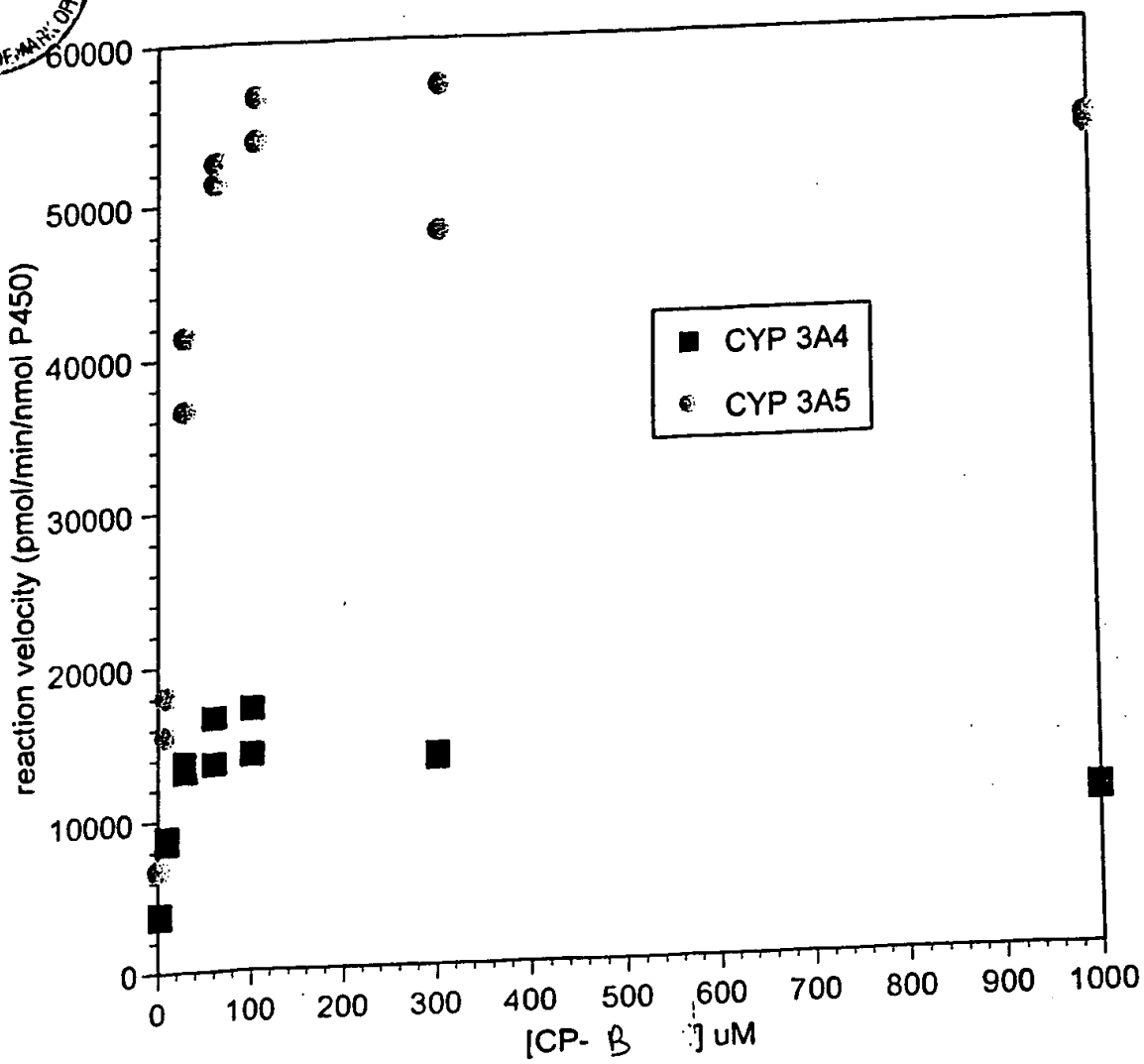

FIGURE 13. SUBSTRATE SATURATION PLOT OF CP B
N-DEALKYLATION BY CYTOCHROME P4503A4 AND 3A5